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SCR 0355P

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cochrane et al.)
Serial No.: 07/715,397) Group Art Unit: 189
Filed: June 14, 1989)
For: PULMONARY SURFACTANT)
PROTEIN AND RELATED)
POLYPEPTIDE)
Examiner: Perkins)

DECLARATION

Hon. Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Charles G. Cochrane, M.D., declare that:

1. I am the first-named inventor of the invention described in the above-identified patent application.

2. Under my direction and control, two synthetic surfactant peptides, designated RL4, having the amino acid residue sequence RLLLLRLLLLRLLLLRLLLLR, and KL4, having the amino acid residue sequence KLLLLKLLLLKLLLLKLLLLK, were synthesized. The peptide sequences correspond to the formula set forth on page 27, line 1 through page 28, line 15, which includes Table 3, of the above-identified application and also correspond to the sequences set forth in the pending claims. Surfactant solutions containing phospholipid and one or the other of the above-noted peptides ("peptide-containing" or "synthetic" surfactants) were prepared according to the teachings of the above-identified application for use in the studies described herein.

3. Under my direction and control, in vivo studies were

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initiated to confirm the efficacy of the novel peptides (and synthetic surfactants containing those peptides) described in the above-noted application. Surfactants consisting of phospholipid and RL4 or KL4 peptides were compared to a phospholipid surfactant not containing peptide or protein ("control surfactant"). Following standard protocols, fetal rhesus monkeys of about 128-131 days gestation were delivered and an endotracheal tube inserted through a tracheostomy.

4. Each monkey was subsequently connected to a ventilator and a catheter was placed in the umbilical artery for the purpose of measuring blood gases and blood pressure; a second catheter was placed in the umbilical vein for the purpose of infusing the monkey with a nutrient/hydrating solution (D10W). After the animals were stabilized, X-rays were taken to assess the presence and extent of respiratory distress syndrome (RDS). Various parameters were adjusted to maintain the oxygen pressure (pO_2) in the range of 50-70 torr and the carbon dioxide pressure (pCO_2) at 45-50 torr. Pulse oximetry was used to continuously monitor hemoglobin saturation of arterial blood.

5. As an index of oxygenation, a/A (arterial/alveolar) O_2 ratios were calculated at the time of each measurement of arterial pO_2 . These values, along with radiographic evidence and clinical assessments of the monkeys' condition, allowed determination of the presence and severity of RDS. An a/A ratio of 0.2 to 0.4 confirms the presence of RDS; values below 0.2 are indicative of severe RDS.

6. Once the diagnosis of RDS was established, each monkey was maintained with ventilatory support, generally for 2 hours. Peptide-containing or control surfactants were then administered via a feeding tube inserted down the endotracheal tube. One-half the dose of synthetic surfactant was given with the animal held on its right side, and the other half while the animal was held on its

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left side. In the experiments illustrated in Figures 3A and 3B (discussed in paragraph 8 below), the individual instilling the synthetic surfactant was not informed as to which surfactant (i.e., peptide-containing surfactant or phospholipid control surfactant) the animal was receiving.

7. Ventilatory support was maintained for another 6-12 hours and each animal's condition was continuously monitored. Mid-experiment and pre-terminal X-rays were taken as well. When the experiment terminated, each animal was sacrificed (by phenobarbital injection) and a necropsy performed.

8. The data generated from the above-described studies are illustrated in Figures 1-4 attached hereto. The Figures illustrate the following.

Figures 1 and 2 - The effect of administration of RL4-containing surfactant on lung function is shown. In Fig. 1, the index of oxygenation (a/A) is plotted against time subsequent to delivery of the animal, in hours. The surfactant was administered in split dosage, as described above, about 28 hours after delivery. In Figure 2, for a second monkey, RL4-containing synthetic surfactant was administered in split dosage, as described above, during the first 2.5 hours after delivery. As in the first monkey (Fig. 1), the a/A ratio dramatically improved in the hours following peptide-containing surfactant administration.

Figures 3A and B - The effect of KL4-containing synthetic surfactant administration on lung function is shown. In Fig. 3A and 3B, the data for eight monkeys are shown; those which were later confirmed to have received KL4-containing synthetic surfactant were identified as Monkey Nos. 6, 7, 8, and 10, while those monkeys receiving another surfactant (i.e., one not containing a surfactant peptide of the present invention) were Monkey Nos. 3, 5, 9, and 11. In all plots, a/A is plotted against hours after birth, with the time of administration of surfactant

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indicated. In Monkey No. 8, the lack of improvement in a/A ratio was found to be the result of an interventricular septal defect allowing right-to-left shunting of oxygen-poor venous blood. Values for final oxygen revealed that the animals receiving peptide-containing surfactant tolerated low concentrations of inspired oxygen; their $p\text{CO}_2$ levels were low, final pH of their blood was normal, and their lungs were expanded as determined by gross and microscopic inspection (the studies were blinded as noted above). In each case, X-rays performed immediately before surfactant administration demonstrated clouding of the lung fields, but only in the four monkeys receiving KL4-containing surfactant did the lung fields clear by 8-10 hours after birth.

Figure 4 illustrates the gradual withdrawal of oxygen over time (in hours), subsequent to administration of KL4-containing surfactant. At time zero and 100% inspired oxygen ($\text{FiO}_2 = 1.0$), the animal was receiving 100% oxygen; at 22-25 hours, the animal was receiving 20% oxygen -- that is, room air.

9. From these data I conclude that the synthetic surfactants of the present invention are not merely therapeutically useful, they produce a dramatic improvement in the recipient's lung function within a relatively brief period of time. This utility is well-illustrated by the fact that animals receiving a synthetic surfactant containing a peptide conforming to the formulations disclosed in the above-referenced application fully recovered from RDS after administration of RL4 or KL4 surfactant. In addition, the data indicate that administration of these novel peptide-containing surfactants allows recovery sufficient to warrant the removal of enriched oxygen administration once the a/A ratio indicates that the organism is no longer experiencing respiratory distress.

10. The experimental results discussed above are consistent with, and predicted by, the information disclosed in the

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specification as filed. In particular, the "pulsating bubble" assays described on pages 42-43 and 48-55 of the specification -- which includes the data shown in Table 7 on pages 53-54 -- provide a valuable *in vitro* model of *in vivo* efficacy. The "bubble" assay results predicted that the synthetic surfactants of the present invention, including, for example, the RL4 tested according to the protocol described herein, would demonstrate therapeutic efficacy, as illustrated herein. In addition, the "bubble assay" results were consistent with, and predictive of, the therapeutic efficacy of various synthetic surfactants verified via the *in vivo* dynamic compliance assays described on pages 55-60 of the specification.

11. The studies described in paragraphs 3-10 were performed in the United States.

12. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

4.8.92

Date

Charles G. Cochrane

Charles G. Cochrane, M.D.

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and trademarks Washington, D.C. 20231 on

4-13-92

(Date of Deposit)

April C. Logan, Reg. No. 33,950

Name of applicant, assignee or
Registered Representative

April C. Logan

Signature

4-13-92

Date of Signature